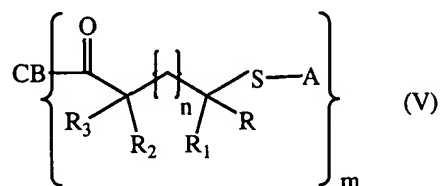


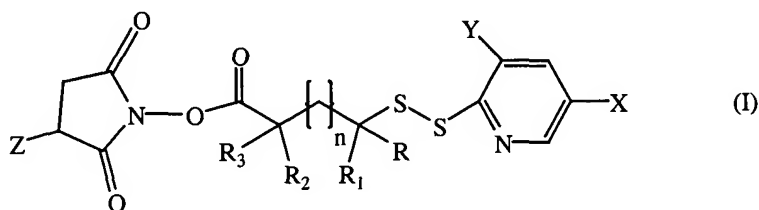
# WHAT IS CLAIMED IS:

1. A method of making a conjugate comprising a cell binding agent and one or more small molecule drugs, wherein said conjugate is represented by formula (V):

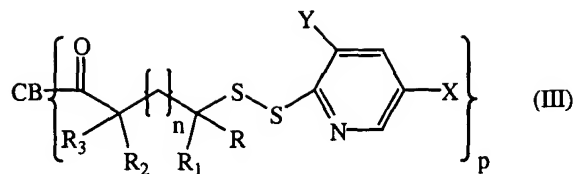


wherein CB represents the cell binding agent, A represents the small molecule drug linked by a disulfide moiety, R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer from 1 to 4, and m is an integer of 1 to 10 or more, said method comprising:

- (1) reacting the cell binding agent with a cross-linker of the formula (I):



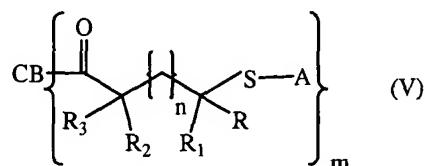
wherein X and Y are the same or different and are H, CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, provided that X and Y are not both H at the same time, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and Z is SO<sub>3</sub><sup>-</sup>M<sup>+</sup> or H, wherein M<sup>+</sup> represents a metal ion or a tetra alkyl ammonium ion, to thereby give a compound of the formula (III):



wherein  $p$  represents an integer of 1 to 10 or more, and

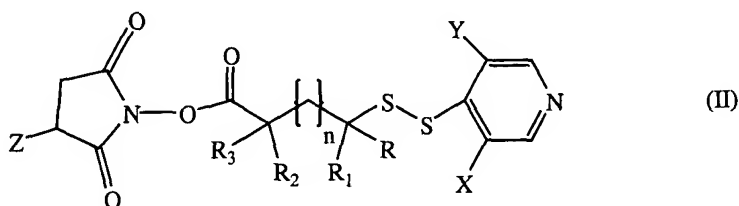
(2) reacting the compound of the formula (III) with one or more small molecule drugs comprising a free thiol group.

2. A method of making a conjugate comprising a cell binding agent and one or more small molecule drugs, wherein said conjugate is represented by formula (V):



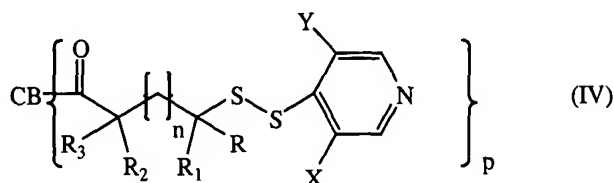
wherein CB represents the cell binding agent, A represents the small molecule drug linked by a disulfide moiety, R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms,  $n$  is 0 or an integer from 1 to 4, and  $m$  is an integer of 1 to 10 or more, said method comprising:

(1) reacting the cell binding agent with a cross-linker of the formula (II):



wherein X and Y are the same or different and are H, CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, provided that X and Y are not both H at the same time, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl,

ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and Z is  $\text{SO}_3\text{M}^+$  or H, wherein  $\text{M}^+$  represents a metal ion or a tetra alkyl ammonium ion, to thereby give a compound of the formula (IV):

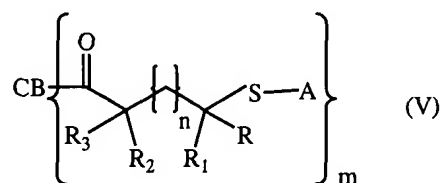


wherein p represents an integer of 1 to 10 or more, and

(2) reacting the compound of the formula (IV) with one or more small molecule drugs comprising a free thiol group.

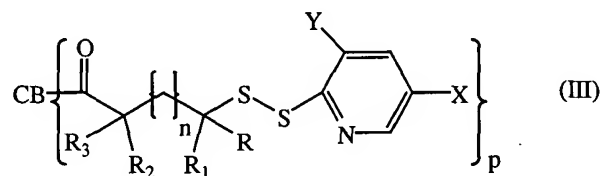
3. The method of claim 1 or 2, wherein the cell-binding agent is an antibody or an antigen binding fragment thereof.
4. The method of claim 1 or 2, wherein the cell-binding agent is a monoclonal antibody or an antigen binding fragment thereof.
5. The method of claim 1 or 2, wherein the small molecule drug is a cytotoxic agent.
6. The method of claim 1 or 2, wherein the small molecule drug is at least one member selected from the group consisting of a maytansinoid compound, a taxane compound, a CC-1065 compound, a daunorubicin compound, a doxorubicin compound, and analogues or derivatives thereof.
7. The method of claim 1 or 2, wherein both of R and  $\text{R}_1$  are H or methyl, or one of R and  $\text{R}_1$  is H and the other is methyl.
8. The method of claim 1 or 2, wherein n is 1,  $\text{R}_1$  is methyl, and R,  $\text{R}_2$  and  $\text{R}_3$  are H.

9. The method of claim 1 or 2, wherein n is 1 and R, R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are H.
10. The method of claim 1 or 2, wherein n is 1, R and R<sub>1</sub> are both methyl, and R<sub>2</sub> and R<sub>3</sub> are both H.
11. A method of making a conjugate comprising a cell binding agent and one or more small molecule drugs, wherein said conjugate is represented by formula (V):



wherein CB represents the cell binding agent, A represents the small molecule drug linked by a disulfide moiety, R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer from 1 to 4, and m is an integer of 1 to 10 or more, said method comprising:

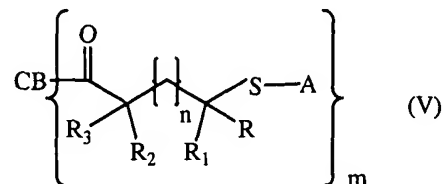
reacting a compound of the formula (III)



wherein X and Y are the same or different and are H, CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, provided that X and Y are not both H at the same time, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and p represents an integer of 1 to 10 or more,

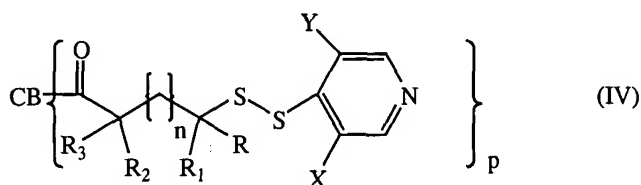
with one or more small molecule drugs comprising a free thiol group.

12. A method of making a conjugate comprising a cell binding agent and one or more small molecule drugs, wherein said conjugate is represented by formula (V):



wherein CB represents the cell binding agent, A represents the small molecule drug linked by a disulfide moiety, R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer of 1 – 4, and m is an integer of 1 to 10 or more, said method comprising:

reacting a compound of the formula (IV):

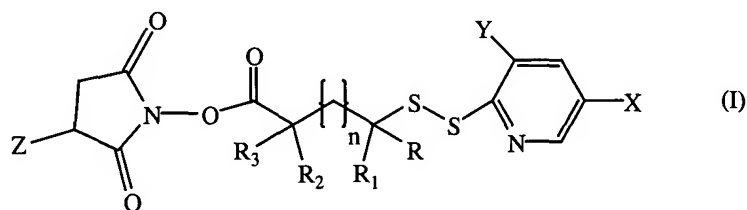


wherein X and Y are the same or different and are H, CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, provided that X and Y are not both H at the same time, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and p represents an integer of 1 to 10 or more,

with one or more small molecule drugs comprising a free thiol group.

13. The method of claim 11 or 12, wherein the cell-binding agent is an antibody or an antigen binding fragment thereof.

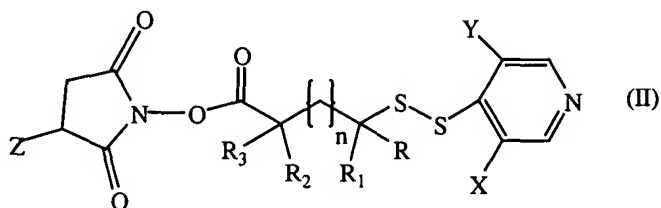
14. The method of claim 11 or 12, wherein the cell-binding agent is a monoclonal antibody or an antigen binding fragment thereof.
15. The method of claim 11 or 12, wherein the small molecule drug is a cytotoxic agent.
16. The method of claim 11 or 12, wherein the small molecule drug is at least one member selected from the group consisting of a maytansinoid compound, a taxane compound, a CC-1065 compound, a daunorubicin compound, a doxorubicin compound, and analogues or derivatives thereof.
17. The method of claim 11 or 12, both of R and R<sub>1</sub> are H or methyl, or one of R and R<sub>1</sub> is H and the other is methyl.
18. The method of claim 11 or 12, wherein n is 1, R<sub>1</sub> is methyl, and R, R<sub>2</sub> and R<sub>3</sub> are H.
19. The method of claim 11 or 12, wherein n is 1 and R, R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub> are H.
20. The method of claim 11 or 12, wherein n is 1, R and R<sub>1</sub> are both methyl, and R<sub>2</sub> and R<sub>3</sub> are both H.
20. A cross-linker of formula (I):



wherein R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer from 1 to 4, X and Y are the same or different and are CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and Z is SO<sub>3</sub><sup>-</sup>M<sup>+</sup> or

H, wherein  $M^+$  represents a metal ion or a tetra alkyl ammonium ion, provided that when X and/or Y is  $NO_2$ , Z is not H.

21. A cross-linker of formula (II):



wherein R,  $R_1$ ,  $R_2$  and  $R_3$  are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer from 1 to 4, X and Y are the same or different and are  $CONR_4R_5$  or  $NO_2$ ,  $R_4$  and  $R_5$  are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and Z is  $SO_3M^+$  or H, wherein  $M^+$  represents a metal ion or a tetra alkyl ammonium ion, provided that when X and/or Y is  $NO_2$ , Z is not H.

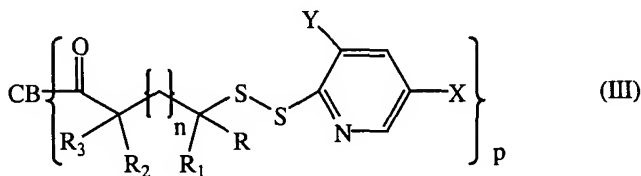
22. The cross-linker of claim 20 or 21, wherein both of R and  $R_1$  are H or methyl, or one of R and  $R_1$  is H and the other is methyl.

23. The cross-linker of claim 20 or 21, wherein n is 1,  $R_1$  is methyl and R,  $R_2$  and  $R_3$  are H.

24. The cross-linker of claim 20 or 21, wherein n is 1 and R,  $R_1$ ,  $R_2$ , and  $R_3$  are H.

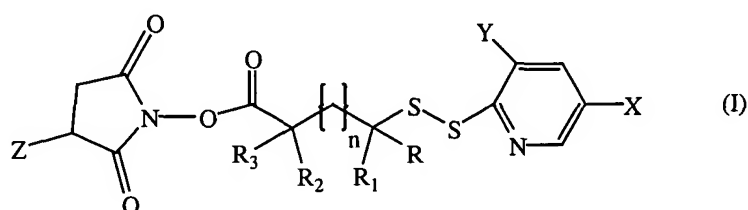
25. The cross-linker of claim 20 or 21, wherein n is 1, R and  $R_1$  are both methyl, and  $R_2$  and  $R_3$  are both H.

26. A method of making a compound of formula (III):



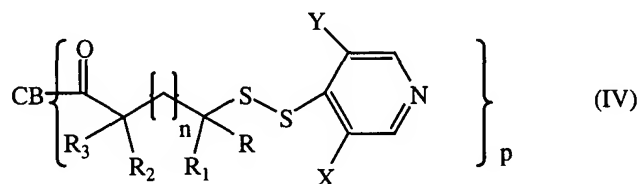
wherein CB represents a cell binding agent, R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer from 1 to 4, X and Y are the same or different and are H, CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, provided that X and Y are not both H at the same time, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and p represents an integer of 1 to 10 or more,

comprising reacting the cell binding agent, CB, with a cross-linker of the formula (I):



wherein Z is SO<sub>3</sub><sup>-</sup>M<sup>+</sup> or H, wherein M<sup>+</sup> represents a metal ion or a tetra alkyl ammonium ion.

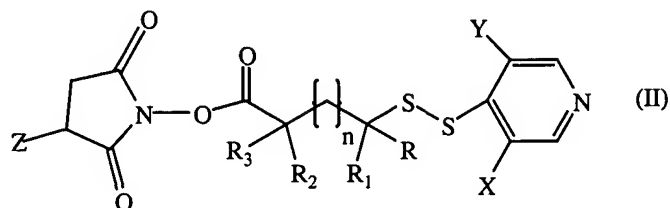
27. A method of making a compound of formula (IV):



wherein CB represents a cell binding agent, R, R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are the same or different and are H, methyl, ethyl, or linear, branched or cyclic alkyl having 3 to 6 carbon atoms, n is 0 or an integer from 1 to 4, X and Y are the same or different and are H, CONR<sub>4</sub>R<sub>5</sub> or NO<sub>2</sub>, provided that X and Y are not both H at the same time, R<sub>4</sub> and R<sub>5</sub> are the same or different and are each H, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, iso-butyl or tert-butyl, and p represents an integer



of 1 to 10 or more,, comprising reacting the cell binding agent with a cross-linker of the formula (II):



wherein Z is  $\text{SO}_3^-\text{M}^+$  or H, wherein  $\text{M}^+$  represents a metal ion or a tetra alkyl ammonium ion.

28. The method of claim 26 or 27, wherein the cell-binding agent is an antibody or an antigen binding fragment thereof.

29. The method of claim 26 or 27, wherein the cell-binding agent is a monoclonal antibody or an antigen binding fragment thereof.

30. the method of claim 26 or 27, wherein both of R and  $\text{R}_1$  are H or methyl, or one of R and  $\text{R}_1$  is H and the other is methyl.

31. The method of claim 26 or 27, wherein n is 1,  $\text{R}_1$  is methyl, and R,  $\text{R}_2$  and  $\text{R}_3$  are H.

32. The method of claim 26 or 27, wherein n is 1 and R,  $\text{R}_1$ ,  $\text{R}_2$ , and  $\text{R}_3$  are H.

33. The method of claim 26 or 27, wherein n is 1, R and  $\text{R}_1$  are both methyl, and  $\text{R}_2$  and  $\text{R}_3$  are both H.